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(54) Title: PROCEDURE FOR TREATMENT OF SEED MATERIAL TO BE GERMINATED

(57) Abstract

The invention concerns a procedure for treating seed material which is to be germinated, to said seed material being added, in conjunction with the germination process, a lactic acid bacteria preparation or a preparation produced by lactic acid bacteria and having an effect inhibiting microbial growth.

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PROCEDURE FOR TREATMENT OF SEED MATERIAL TO BE GERMINATED

FIELD OF THE INVENTION

The present invention concerns a procedure for treating seed material intended to be germinated.

BACKGROUND OF THE INVENTION

A germination process is in this context understood to be a process step in general which is required in order to produce a germinated product, starting from storage-dry seed. In the brewery and distillery industry, for instance, a germination process, that is the malting process, is applied in producing the important raw material of beer, or other alcoholic beverages, viz. cereal malt, such as barley malt, rye malt or any malt whatsoever. The germination process is furthermore applied in producing various commercial sprouts products, e.g. bean sprouts or any sprouts for use in human nutrition.

Germination processes are usually carried out in non-aseptic conditions. On the seeds being treated there occur microbes originating from the growth environment or from storage. Conditions during the germination process are mostly favourable to the microbes present on the seeds, such microbes usually multiplying during the course of the process. The microbes may exert a detrimental effect on the germinated product, or on the end product which may ultimately be made thereof, while they may equally have beneficial influence on the product being germinated.

The main steps in brewing process are: malting, wort production, primary and secondary fermentation, and downstream processing. The purpose with malting is to produce in the kernel enzymes which in the

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mashing step decompose the substances of the kernel's endosperm to a form soluble in the wort. For example, the barley seed malting process is well known to comprise three steps; steeping, germination and kilning. The cleaned and screened barley grains are steeped in water until desired moisture is achieved, e.g. on the order of 43 to 44%. Part of the steeping may be accomplished in so-called air rest. The barley is allowed to germinate in controlled conditions, and the germinated barley is kilned in a hot air current until the germination has come to an end. On termination of kilning, the rootlets are removed from the malt. Regulation of the malting steps is based on temperature, air flow and moisture/humidity control.

Malt quality is affected, on the side of malting technique and malting conditions, also by the microbial flora of the malt cereal, this flora varying significantly e.g. depending on cereal variety, weather conditions, growth site, length of growing season and storage conditions.

Barley is the cereal most often used in malting. The inherent, natural microbial flora of barley can be classified as field and storage fungi, bacteria and yeasts. The commonest field fungi of barley are: Fusarium, Alternaria, Cladosporium, Cephalosporium, Epicoccum, and Helminthosporium. The occurrence of moulds is different in different countries and different years. Wet weather conditions during the cereal's growth period, and particularly while it is being harvested, favour the growth of Fusarium mould. Fusarium contamination may be heavy indeed in rainy growth seasons. One of the commonest bacterium species on cereals is Enterobacter agglomerans. Other bacteria which should be mentioned are; Escherichia coli, and bacteria of genera Pseudomonas, Micrococcus and Bacillus, and lactic acid bacteria. The bacterial count on barley is about 10^5 to 10^8 CFU/g (colony forming units per g).

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The moulds and bacteria in barley increase during malting, and peak concentrations are usually reached during the germination step. Fusarium moulds, in particular, and lactic acid bacteria undergo the strongest proliferation. Yeasts also increase during malting. In the kilning step the mould, yeast and bacterium concentrations go down again, as a rule. Part of the microbes present on barley have a useful effect in view of malting and of the product to be made of the malt, e.g. beer. It has been estimated that up to 40-50% of some enzymes in the malt are of microbial origin. On the other hand, part of the microbes exert a detrimental influence on the barley and/or the malts.

Among the disadvantageous microbes Fusarium moulds deserve to be mentioned, which have been found to cause particularly detrimental gushing of beer more often than other moulds, the peptides produced by said moulds constituting nuclei for gas bubbles discharging from the beer bottle in the form of powerful gushing. When more than 50% of malted kernels are contaminated with Fusarium moulds, the risk of gushing is clearly increased.

Further, gram-negative bacteria present in the barley, such as species belonging to genera Pseudomonas and Flavobacterium, and gram-positive bacteria of genus Leuconostoc have been shown to retard filtration of the mash in connection with wort production. Various microbes present in barley may also give rise to other disadvantageous effects, e.g. inhibit the germination, cause off-flavours or unfavourable changes in the analysis values of the wort and the beer.

The quality requirements of malt barley can be specified in annually established cultivation contract and delivery terms. Moulds are a group of microbes frequently mentioned in quality specifications. Many malting plants have moreover imposed an upper limit on certain moulds. If the proportion of Fusarium-contaminated

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kernels exceeds 65% or if the corresponding proportion of Aspergillus and Penicillium moulds exceeds 50%, the barley can be classified as poor in quality or even as unfit for use in malting.

Attempts have been made to prevent gushing induced by moulds, by using barley of good quality or by blending barley, malt or beer batches. In rainy years nearly the whole barley crop may be poor in quality, in which case it may be impossible to obtain good barley. Microbicidic chemicals have also been tried in order to reduce the quantity of moulds, but no safe and generally approved chemical could be found.

A germinating process for producing sprouts intended to be used for nutrition offers likewise propitious conditions for proliferation e.g. of moulds and bacteria. Such sprouts products will spoil rapidly. Further still, in connection with germination increase may take place e.g. of foodstuff pathogens causing food poisoning, such as Salmonella, Yersinia and/or Listeria bacteria.

SUMMARY OF THE INVENTION

The object of the present invention is to eliminate the drawbacks just discussed.

Specifically, an object of the invention is to provide a procedure by which the quantity and quality of the microbial flora can be carefully regulated during the germinating process, however without disadvantageously affecting the quality of the germinated product or of the end product potentially made of the product.

Regarding the features characterizing the invention, reference is made to the claims.

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DETAILED DESCRIPTION OF THE INVENTION

The invention is based on studies in connection of which the unexpected observation was made that lactic acid bacteria can be used towards improving the quality of a product to be germinated. The substances comprised and/or produced by the preparation of the invention, i.e., the microbicidic agents, inhibit the growth of detrimental microorganisms occurring in connection of a germinating process.

The use of lactic acid bacteria in foodstuff and animal feed industry is well known in the art. They produce in fermentative conditions such compounds which affect the composition and flavour of the products, but which also inhibit the growth of pathogenic microbes tending to spoil said products. Lactic acid bacteria have been commonly used in dairy products, meat products, vegetable fermentation and bakery products, and in fodder preservation.

Addition of lactic acid bacteria or of lactic acid has been practiced in the production of a certain malt type, of so-called Sauermalz. This addition is made to the malt in the kilning step, prior to mashing or during mashing. The purpose with the addition is merely to cause lowering of the wort pH and, thus, to exert an influence on the course of the mashing process and on the quality of the finished beer. However, lactic acid bacteria have not heretofore been used as taught by the invention: to inhibit the growth of undesired microbes in connection with the germinating process.

The preparation of the invention can be added to the product to be germinated in any step of the germinating process.

In a particularly advantageous embodiment, lactic acid bacteria preparation, or preparation produced by a lactic acid bacteria, is added to cereal material, such as barley kernels, during the course of

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the malting process. The preparation added inhibits the growth of moulds, in particular of harmful Fusarium moulds, and of bacteria, as a result of which, for instance, the risk of beer gushing due to Fusarium mould is reduced. However, the preparation has no substantial effect on the action of the useful microbial flora as regards the quality of the malt that is obtained or of the beer therefrom produced. No harmful effects of the preparation added on malt quality have been observed either, nor has it been found to contain or to produce compounds which are harmful in view of the malt, or beer, being produced.

In a malting process, the lactic acid bacterium preparation, or the preparation produced by a lactic acid bacteria, can be added to the barley kernels before steeping, in the steeping step or in the germination step. The addition is advantageously made in the steeping or germinating step. The malting process may be carried out, in other parts, in any manner known in itself in the art. If desired, e.g. nutrients may be added to the barley to be malted, or the conditions may be regulated, e.g. lactic acid added, in order to optimize the conditions for growth of lactic acid bacteria.

It is possible in the invention to use any commonly available lactic acid bacterium whatsoever which possesses influence of inhibiting microbial growth. The following usable lactic acid bacterium genera may be mentioned: Lactococcus, Leuconostoc, Pediococcus and Lactobacillus. The following may be mentioned to present advantageous species: Lactococcus lactis, Leuconostoc mesenteroides, Pediococcus damnosus, Pediococcus parvulus, Pediococcus pentosaceus, Lactobacillus curvatus, and Lactobacillus plantarum, or any mixtures of these, among these the following being particularly advantageous: Lactobacillus plantarum and Pediococcus pentosaceus or mixtures thereof. Use of genetically modified lactic acid bacteria is equally

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possible.

The lactic acid bacteria preparation may be composed of culture broth, with or without cells, or of concentrated culture broth (including cells). A preparation produced by lactic acid bacteria may consist of cell-free culture filtrate, of concentrated culture filtrate, of fractionated culture filtrate, or of a pure or partly purified microbicidic product.

According to a particularly advantageous embodiment, the treatment is carried out with concentrated or fractionated culture broth, which may be cell-free or may contain cells. Concentration may be accomplished e.g. by lyophilization or by evaporation. The culture broth is concentrated e.g. by a factor of 2 - 20 - 40.

Fractionation, i.e., purification of the microbicidic products, can be carried out in a manner known in itself in the art, e.g. with the aid of chromatographic methods or by ultrafiltration.

In the procedure of the invention, the microbial growth-inhibiting activity of the preparation containing lactic acid bacteria, or of preparation produced by lactic acid bacteria, to be added to the seed material corresponds e.g. to the culture broth quantity of about 10 to 10,000 ml/kg of seed material to be treated, suitably 30 to 7,000 ml/kg of seed material to be treated, e.g. 40 to 5,000 ml/kg of seed material to be treated. The preparation of the culture broth is described in the Examples section. It should be noted that in the application in hand the activity of the preparation is defined with the aid of the culture broths employed. It is obvious to a person skilled in the art that preparations according to the invention possessing equivalent microbial growth-inhibiting activity can equally be produced applying other culture broths and/or procedures.

According to the invention the preparation

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contains microbicidic compounds, and/or the preparation produces microbicidic compounds during the course of the germination process. When a cell preparation is employed, cell growth can be promoted, if required, e.g. by regulating the conditions during the germination process, or by adding nutrients. The preparation may also accelerate the growth of other lactic acid bacteria present in the material to be germinated.

Since the use of lactic acid bacteria in foodstuffs is allowed and generally approved, the preparation derived from lactic acid bacteria growing is also safe to use. Lactic acid bacteria belong usually to the natural microbe flora of seeds to be germinated, such as barley kernels. Therefore the procedure of the invention is maximally natural. It is also possible to use for lactic acid bacterium strain a strain inherently occurring on the seeds.

Thanks to the invention, it becomes possible in malting to reduce the detriments arising from Fusarium contaminations, such as gushing of beer.

Moreover, the procedure has unexpectedly been found to improve the filtrability characteristic in the brewing process. This has been found to be due to the fact that the preparation of the invention also restricts the counts of harmful species occurring in malting and retarding the filtration of the mash, e.g. those belonging to genera Leuconostoc, Pseudomonas and Flavobacterium.

According to another advantageous embodiment, lactic acid bacteria preparation or preparation produced by a lactic acid bacterium is added to the seed material when producing sprouts to be used for food.

Thanks to the present invention, the growth of harmful microbes can be restricted in connection with a germination process. For the first time, the invention enables biological expedients to be used in order to prevent the growth during an industrial germination

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process of detrimental bacteria occurring on seed which is to be germinated.

The procedure of the invention improves the general hygienic standard of the germination process even on the whole.

In the following the invention shall be illustrated with embodiment examples, which are merely intended to illustrate the invention, without confining it to them. The treatment of the invention is also applicable in other germination processes.

Fig. 1 shows a graph representing the microbicidic activity in a lactic acid bacteria culture filtrate, as assessed by the turbidometric method. The normal growth curve of the test organism, E.agglomerans E-396, and the inhibition effect exerted on the growth of the test organism by culture filtrates of the production strains L. plantarum E-76 and P. pentosaceus E-390.

In Fig. 2 is shown the effect on the total bacterial counts of the malting of P. pentosaceus E-390 culture broth added at different stages of the maltings.

In Fig. 3 is shown the effect on the total bacterial counts of the malting of P. pentosaceus E-390 cells added at different stages of the maltings.

In Fig. 4 are shown the total bacterial counts at different stages of laboratory maltings on addition of P. pentosaceus E-390 and L. plantarum E-76 culture broths, or concentrated culture broths, to the barley steeping water.

In Fig. 5 are shown the total bacterium counts at different stages of laboratory maltings on addition of P. pentosaceus E-390 and L. plantarum E-76 culture broths, or concentrated and fractionated culture broths, to the barley steeping water.

Fig. 6 displays the effect of lactic acid bacteria cultures, added to the malting, on mash filtrati-

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on (Tepral filtration).

Example 1: THE MICROBICIDIC EFFECT OF VARIOUS LACTIC ACID BACTERIA STRAINS ON MICROBES OCCURRING IN MALTING

In the experiment, the microbicidic effect on microbes occurring in malting exerted by preparations produced by various lactic acid bacteria strains were studied. The sterile-filtrated culture broth was used for preparations.

1. Production strains:

The following lactic acid bacteria strains were used for production strains:

Lactobacillus lactis		
ssp. lactis	VTT-E-90414	(E-414)
ssp. diacitilactis	VTT-E-90423	(E-423)
Leuconostoc mesenteroides		
ssp. mesenteroides	VTT-E-90389	(E-389)
ssp. mesenteroides	VTT-E-90415	(E-415)
ssp. mesenteroides	VTT-E-90466	(E-466)
Pediococcus damnosus	VTT-E-76065	(E-65)
Pediococcus parvulus	VTT-E-88315	(E-315)
Pediococcus pentosaceus	VTT-E-76067	(E-67)
Pediococcus pentosaceus	VTT-E-76068	(E-68)
Pediococcus pentosaceus	VTT-E-88317	(E-317)
Pediococcus pentosaceus	VTT-E-90390	(E-390) (DSM 7389)
Lactobacillus curvatus	VTT-E-90391	(E-391)
Lactobacillus plantarum	VTT-E-78076	(E-76) (DSM 7388)
Lactobacillus plantarum	VTT-E-79098	(E-98)

The strains were obtained from the VTT, Collection of Industrial Microorganisms (Biotechnical Laboratory, Finland). Lactobacillus plantarum (E-76) has been deposited with DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen) under number 7388. The strain E-76 (DSM 7388) had been isolated from beer

BNSDOCID: <WO___9416053A1_I_>

by known techniques used for liquid products, and analyzed/identified using well known analysis methods. Pediococcus pentosaceus (E-390) has been deposited with DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen) under number 7389. The strain E-390 (DSM 7389) had been isolated from homogenized samples of split barley kernels and identified/analyzed using techniques well known in the art [see the article of Haikara, A. and Home, S., Mash Filtration difficulties caused by split barley kernels: a Microbiological problem, in the publication of EBC Congress 1991 (Quality Control)]. The deposits are under the provisions of the Budapest Treaty.

2. Test strains:

Various harmful microbial species occurring at malting were used for test strains, as well as lactic acid bacteria strains which served as production strains, among others.

Harmful moulds were represented in the tests by Fusarium moulds [Gibberella avenacea (former Fusarium avenaceum) VTT-D-80141 (D-141) and VTT-D-80147 (D-147), and Fusarium culmorum VTT-D-80148 (D-148) and VTT-D-80149 (D-149), Collection of Industrial Microorganisms, Biotechnical Laboratory of VTT], and one Aspergillus species.

Harmful gram-negative bacteria were represented by two strains from genus Enterobacter and by one species each from genus Flavobacterium and genus Pseudomonas. The lactic acid bacteria consisted of those strains which were employed as production strains, plus the strain Lactococcus sp. E-416.

3. Cultivation of production strains, and preparation of sterile filtered culture broth:

The lactic acid bacteria were cultivated in MRS broth (MRS BROTH, Oxoid). The Pediococcus strains E-65, E-67 and E-68 were aerobically cultivated at 25°C, all other production strains anaerobically at

BNSDOCID: <WO___9416053A1_I_>

30°C, cultivation period varying from 2 to 5 days. The cells were then centrifuged and the supernatant was sterile-filtered.

4. Cultivation of test strains:

Spore suspensions were produced from the Fusarium moulds by cultivating the mould strain in CMC (carboxymethyl cellulose) solution at 25°C, 5 to 6 days as a shaking culture, dispersing the spore formations in TWEEN solution, filtering the suspension and recovering the filtrate.

The spore suspension of Aspergillus mould was produced directly on PD agar (25°C, 3 days) (Potato dextrose, Difco).

Gram-negative bacteria were aerobically cultivated in NB broth (Nutrient broth, Difco) for 1 day, the Enterobacter strain at 30°C, and the Flavobacterium and Pseudomonas strains at 25°C.

Lactic acid bacteria were cultivated as described under 3. above.

5. Examination of the microbicidic effect of the lactic acid bacteria:

The microbicidic activity in the culture broth was assessed by the disk method, or turbidometrically.

5.1. Disk method for assessment of microbicidic activity:

Sterile-filtered culture broth, or a dilution thereof, was pipetted onto a filter paper disk (12.7 mm dia.), 100 µl. The disks were placed on Plate Count agar dishes into which 0.3 ml of test organism dilution, 10^{-2}, had been pour plated. The specimens were cultivated for 24 hrs at 30°C, whereupon the diameter in mm of the inhibition zone that had formed was measured.

5.2. Turbidometric method for assessment of microbicidic activity:

An automatic turbidometer (Bioscreen, Labsys-

BNSDOCID: <WO___9416053A1_I_>

tems) was employed in the procedure.

The sample contained 10% by vol. of the test organism and 10% by vol. of the sterile-filtered culture broth of the production strain, calculated on the sample volume, and growth medium. For controls, the sterile filtrate preparation was replaced with distilled water of which the pH had been adjusted with lactic acid to identical level with the sterile-filtered preparation.

The growth medium used was, in the case of each test strain, the same medium as in the test strain cultivation.

The growth conditions for Fusarium and Aspergillus moulds were: 5 days, 25°C, powerful shaking. Those for gram-negative bacteria were: Enterobacter strain, 30°C, all others 25°C, 2 days, and shaking. The growth conditions for lactic acid bacteria were: 3 days, 30°C, and shaking.

The apparatus determined from the samples the absorbance at visible light wavelength 420-580 nm. After cultivation, the growth curve of each sample could be made up and the area subtended by the curve calculated.

The microbicidic effect of a production strain on the growth of the test strain was expressed by an inhibition percentage, obtained by comparison of the growth area sizes found with the control and with the sterile-filtered preparation of the production strain, respectively.

6. Examination of the fungicidic effect of certain lactic acid bacteria:

The fungicidic effect of six lactic acid bacteria strains, E-76, E-98, E-315, E-317, E-414 and E-415, separately on all Fusarium moulds employed as test strains was studied. In the test, a visual examination was made of turbidity formation in mould cultures to which sterile-filtered preparation from each

BNSDOCID: <WO___9416053A1_I_>

lactic acid bacteria cultivation had been added at various dilutions, stated in Table 3.

In the controls, sterilized Milli-Q water and Milli-Q water adjusted with lactic acid to pH 3.6 was used instead of culture broth.

Cultivation took place as a test tube culture in CMC broth, at 25°C, during 5 days. The results were visually read.

7. Results:

Tables 1 and 2, and Fig. 1, display the microbicidic activities determined for the different lactic acid bacteria strains by the disk method and by the turbidometric method, respectively.

Table 3 displays the visually determined fungicidic activities.

TABLE 1. Microbicidic activities of lactic acid bacteria culture broths, by the disk method.

Lactic acid bacterium	Cell count, CFU/ml	Culture broth, pH	inhibition zone dia., mm
E-76	3.2×10^8	3.70	19
E-98	1.2×10^8	3.72	17
E-315	1.6×10^8	3.90	16
E-317	1.0×10^8	3.86	16
E-390	1.9×10^8	3.92	15

Cell count of test organism E-396 - 4.0×10^7 CFU/ml; on dish - 1.2×10^5 CFU/ml.

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TABLE 2. Microbisidic effect of lactic acid bacteria on the growth of various microbes.

Test strain		Production strain													Cell conc.
		Lactococcus		Leuconostoc		Pedicoccus						Lactobacillus			
		E-414	E-423	E-389	E-466	E-65	E-315	E-67	E-68	E-317	E-390	E-391	E-76	E-98	CFU/ml
Moulds															
Aspergillus niger	D-5	-28	-36	-39	-38	-16	-33	-21	-20	-24	-28	-44	-39	-41	$2,0x10^4$
Fusarium avenaceum	D-141[1)]	-36	-36	-49	-55	-33	-59	-42	-44	-52	-47	-42	-79	-74	$6,0x10^3$
" "	D-147[1)]	-12	-20	-36	-66	-8	-63	-27	-43	-50	-53	-37	-70	-67	$2,0x10^4$
" culmorum	D-148[1)]	-5	-17	-29	-26	-9	-25	-10	-16	-20	-24	-21	-35	-30	$2,5x10^3$
" "	D-149[1)]	3	-6	-20	-30	-10	-26	-18	-9	-20	-23	-9	-39	-32	$6,0x10^2$
Gram-negative bacteria															
Enterobacter agglonerans	E-396	-89	-92	-94	ND	-96	-92	-93	-96	-91	-93	-93	-93	-93	$6,0x10^4$
Flavobacterium sp.	E-399[2)]	-98	-99	-99	-98	-99	-97	-98	-98	-98	-98	-99	-96	-97	$2,6x10^6$
Pseudomonas fluorescens	E-397[2)]	-98	-98	-97	-98	-97	-97	-98	-98	-97	-97	-97	-98	-97	$1,0x10^4$
Lactic acid bacteria															
Lactococcus lactis ssp. lactis	E-414	-37	-33	-24	-47	-46	-57	-39	-48	-53	-44	-41	-56	-56	$5,0x10^4$
" ssp.diacitilactis	E-423	-15	-29	-35	-28	-43	-52	-39	-87	-37	-33	-34	-43	-42	$4,0x10^4$
Lactococcus sp.	E-416	-15	-23	-16	-61	-37	-41	-31	-35	-36	-41	-31	-57	-58	$2,0x10^4$
Leuconostoc mesenteroides ssp. mesenteroides	E-389[2)]	-19	-30	-26	-50	-39	-45	-11	-38	-47	-41	-38	-57	-58	$4,0x10^4$
Pedicoccus parvulus	E-315	6	-6	-7	-7	-7	-8	-6	-23	-8	-7	-7	-12	-12	$1,0x10^5$
" pentosaceus	E-68	10	19	21	15	4	20	10	8	9	3	17	18	5	$6,0x10^4$
" "	E-390	0	-3	-1	-7	-6	-5	-3	-6	-5	-8	-5	-7	-9	$2,0x10^5$
Lactobacillus plantarum	E-76	-2	-6	-5	-12	-9	-14	-12	-10	-16	-14	-10	-22	-22	$2,0x10^5$
" "	E-98	-1	-3	-3	-8	-9	-6	-10	-6	-4	-6	-4	-8	-11	$1,0x10^5$

ND: not determined; negat. number: inhibits growth; posit. number: stimulates growth
>55% strong inhibition; 35-55% fairly strong inhibition; 15-34% fairly weak inhibition; 15% weak or no inhibition
[1)]inducing beer gushing; [2)]retards mash filtration

TABLE 3. Effect of lactic acid bacteria culture broth Fusarium fungi causing gushing of beer.

+ distinct growth; - no growth

D-141	Lactic acid bacterium strain	CULTURE BROTH Dilution					
		1:6	2:6	3:6	4:6	5:6	6:6
	E-76	+	+	-	-	-	-
	E-98	+	+	-	-	-	-
	E-315	+	+	+	-	-	-
	E-317	+	+	+	-	-	-
	E-414	+	+	+	+	-	-
	E-415	+	+	+	-	-	-
	Control	+					
	pH control	+					

D-147	Lactic acid bacterium strain	CULTURE BROTH Dilution					
		1:6	2:6	3:6	4:6	5:6	6:6
	E-76	+	+	-	-	-	-
	E-98	+	+	-	-	-	-
	E-315	+	+	+	-	-	-
	E-317	+	+	+	-	-	-
	E-414	+	+	+	+	-	-
	E-415	+	+	+	-	-	-
	Control	+					
	pH control	+					

D-148	Lactic acid bacterium strain	CULTURE BROTH Dilution					
		1:6	2:6	3:6	4:6	5:6	6:6
	E-76	+	-	-	-	-	-
	E-98	+	+	-	-	-	-
	E-315	+	+	-	-	-	-
	E-317	+	+	-	-	-	-
	E-414	+	+	+	-	-	-
	E-415	+	+	-	-	-	-
	Control	+					
	pH control	+					

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D-149	Lactic acid bacterium strain	CULTURE BROTH Dilution					
		1:6	2:6	3:6	4:6	5:6	6:6
	E-76	+	+	-	-	-	-
	E-98	+	+	-	-	-	-
	E-315	+	+	+	-	-	-
	E-317	+	+	+	-	-	-
	E-414	+	+	+	-	-	-
	E-415	+	+	+	-	-	-
	Control	+					
	pH control	+					

The results reveal that the lactic acid bacteria strains mentioned inhibit the growth of harmful Fusarium moulds and other detrimental microbes occurring in the malting process, exerting substantially no influence on useful microbes. The results demonstrate the usability of lactic acid bacteria in the procedure of the present invention.

Example 2: THE MICROCIDIC EFFECT OF CERTAIN LACTIC ACID BACTERIA STRAINS ON FOODSTUFF PATHOGENS AND ON MICROBES DETRIMENTAL TO FOODSTUFFS

In the experiment a study was made of the microcidal effect on foodstuff pathogens and on microbes detrimental to foodstuffs of preparations produced with the aid of Pediococcus pentosaceus VTT-E-90390 (E-390) and Lactobacillus plantarum VTT-E-78076 (E-76) strains. As test organisms, strains belonging to genera Bacillus, Yersinia, Listeria, Pseudomonas, Salmonella and Staphylococcus were employed.

As a preparation, a sterile culture broth of lactic acid bacteria, prepared according to Example 1, was used. All the other test strains were cultivated for 16 to 18 hrs in Iso-Sensitest broth (Oxoid) except the Listeria strain, which was grown in tryptose/phos-

phate broth. Cultivating temperature was 30°C. except for the Salmonella, Listeria and Staphylococcus strains, 37°C.

The microbicidic activity was determined by the turbidometric method, described in Example 1. Experimental conditions, as regards growth substrate and temperature, were as described in the foregoing. The incubation time was 24 hrs, except for Bacillus and Yersinia strains, 48 hrs.

The results are shown in Table 4, which reveals that addition of the lactic acid bacteria preparation of the invention causes inhibition of the growth of foodstuff pathogens and microbes detrimental to foodstuffs.

TABLE 4. Growth inhibition caused by P. pentosaceus E-390 and L. plantarum E-76.

Production strains / Test organisms	E-390	E-76
	Reduction of growth area, %	
Bacillus cereus ATCC91339 *)	93	80
Yersinia enterolitica ELI351 *)	85	54
Listeria monocytogenes KTL4126 *)	41	49
Pseudomonas fluorescens ELI97	59	97
Pseudomonas fragi ATCC4973	84	93
Salmonella infantis ELI5 *)	90	98
Staphylococcus aureus EL1200 *)	73	86

*) Pathogens occurring in foodstuffs
ATCC: American Type Culture Collection
ELI: VTT, Food Research Laboratory
KTL: National Public Health Institute

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Example 3: THE EFFECT OF LACTIC ACID BACTERIA PREPARATIONS AND OF PREPARATIONS PRODUCED BY LACTIC ACID BACTERIA ON THE MICROFLORA OF MALTING AND ON MALT QUALITY

1. Strain employed:

The lactic acid bacteria strain Pediococcus pentosaceus VTT-E-90390 (E-390) was used in the experiment.

MRS broth was used for growth medium of inoculum. The bacteria were anaerobically cultivated for 2 days in 10 ml MRS broth, temperature 30°C. The inoculation volume was 1% of the growth solution volume.

2. Barley:

Kymppi barley of the harvest year 1990 was used, in which the proportion of Fusarium mould-contaminated kernels was 55%.

3. Malting process:

1000 g barley batches were rinsed in a water bath at 12°C for 1 hr. The rinsing water was replaced with the first steeping water, and this was replaced with the second steeping water after 5 hrs. Air rest was commenced 16 hrs thereafter. The purpose with air rest was to eliminate the water on the surface of the kernels. Its duration was 8 hrs. During the steepings, the barley attained 44% moisture. The barley was aerated throughout the steeping process.

Steeping was succeeded by germination. The barley was germinated in a germination boxes for 6 days, at 14°C. In order to maintain the moisture on the 44% level, the barley batches were moistened and turned every day. The green malt thus obtained was dried in a 21-hour temperature programme. The temperature was 50°C for 4.5 hrs. During the next 4.5 hrs it was raised to 60°C, where it was held 4 hrs. The temperature was further raised uniformly during 5 hrs, up to 85°C and held there the remaining 3 hrs. The ultimate moisture content of the malt became about 4%. Finally, the rootlets were mechanically removed.

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A malting run without any additions whatsoever served as control.

4. Lactic acid bacteria preparation and preparation produced by lactic acid bacteria:

Lactic acid bacteria cells isolated from the culture broth and culture broths containing microbicidal compounds were employed for preparations, in combination as well as separately. Culture broth including cells was added 120 ml per kg of barley, or the cells were separated from 120 ml of culture broth. This separation was done by centrifuging the culture broth, and the cells were suspended in water. In the cases in which culture broth was added, the culture broth was used as such. The cell counts of the preparations added were on the order of about 10^8 to 10^9 CFU/ml.

5. Additions of lactic acid bacteria preparation:

The additions of lactic acid bacteria preparation were made either to the barley, to the beginning of steeping I, to the beginning of steepings I and II, or to the beginning of germination.

6. Analyses performed:

Samples were drawn from each malting step.

6.1 Moulds were assessed as follows. The percentage of kernels contaminated with Fusarium moulds was determined by means of CZAPEK IPRODION DICLORAL agar (CZID agar), which is selective to Fusarium moulds, and a moist filter paper (EBC-Analytica Microbiologica, Part II, 1987). Fusarium moulds were identified by their typical colony and spore morphology and by the red colour.

Aspergillus and Penicillium moulds were assessed using selective malt salt agar (EBC-Analytica Microbiologica, Part II, 1987). Other most common moulds were assessed on moistened filter paper.

6.2 Lactic acid bacteria were assessed on MRS agar in the case of cultures added as well as malting

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samples.

6.3 The total bacterial counts were assessed on Plate Count agar (Difco).

6.4 Chemical characteristics of the malt were determined employing methods known from the context of malting (EBC-Analytica, 1987, 4th Ed.).

7. Results:

In Table 5 are given the counts of Fusarium moulds as well as lactic acid bacteria in the different malting steps on addition of E-390 culture broth.

In Figs 2 and 3 are presented the total bacterial counts in different malting steps for E-390 culture broths and for E-390 cells.

Table 6 presents the results of malt analysis on addition of E-390 culture broth or E-390 cells.

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TABLE 5. Effect of P. pentosaceus E-390 culture broth added at different salting steps, on Fusarius and lactic acid bacteria counts during salting.

	Fusarium moulds (percentage of contaminated kernels)			
Addition step	Barley	Steeping	Germination	Malt
Control	55	72	96	25
120 ml CB*) to barley	3	6	77	3
120 ml CB*) to 1st. st. water	55	62	98	14
120 ml CB*) to 1. + 2. st. water	55	42	90	9
120 ml CB*) to germination	55	78	91	3
	Lactic acid bacteria (cell density, CFU/g)			
Control	$6,0x10^1$	$3,4x10^2$	$2,2x10^4$	$3,0x10^3$
120 ml CB*) to barley	$1,2x10^8$	$1,1x10^8$	$2,2x10^8$	$1,8x10^8$
120 ml CB*) to 1st st. water	$6,0x10^1$	$3,1x10^7$	$2,8x10^7$	$1,4x10^7$
120 ml CB*) to 1. + 2. st. water	$6,0x10^1$	$7,4x10^7$	$8,3x10^7$	$5,3x10^7$
120 ml CB*) to germination	$6,0x10^1$	$1,5x10^8$	$2,7x10^8$	$2,6x10^8$

*) CB; culture broth

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TABLE 6.Effect of culture broth or cells of P. pentosaceus E-390 added at various steps of salting on salt quality.

Analysis / Sample	ß-glucans mg/l	Moisture %	Extract %d.m.	Coarse grind %d.m.	Extract differ. %d.m.	Clarity	Filtration fine grind ml/h	Saccharific. time min.	Viscosity cP	FAN mg/l	Modification %	Homogeneity %	α-amylase activity U/g	ß-glucanase activity U/kg	Rootlets g/kg
Control	604	4,1	80,1	77,8	2,3	clear	300	<10	1,59	163	82	69	202	365	35
120 ml CB*) to barley	496	4,3	80,3	77,3	3,0	clear	305	<10	1,51	164	80	57	207	355	37
120 ml CB*) to 1st st. water	482	4,2	80,5	77,5	2,9	clear	310/56 min	<10	1,50	171	76	51	194	316	30
120 ml CB*) to 1+2 st. water	482	4,3	80,0	76,9	3,1	clear	305	<10	1,49	168	80	64	186	360	31
120 ml CB*) to germination	955	4,2	79,0	74,9	4,1	opal.	310	<10	1,68	129	69	59	159	386	21
Cells (10^8 CFU/g) to barley	606	4,3	80,3	77,4	2,9	clear	315/61 min	<10	1,54	167	79	59	223	359	38
Cells (10^8 CFU/g) to 1st st. water	-	-	-	-	-	-	-	-		-	79	59	187	376	39
Cells (10^8 CFU/g) to 1+2 st. water	-	-	-	-	-	-	-	-	-	-	81	67	178	353	36
Cells (10^8 CFU/g) to germination	804	4,3	79,5	76,6	2,9	opal.	290	<10	1,63	148	81	69	179	392	35

- not analysed

*)CB; culture broth

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The results here obtained reveal that treatments according to the invention reduce in particular the Fusarium mould quantity and the total bacterial count in different steps of malting.

Addition of preparation had no detrimental effect on malt quality. Quite the opposite is true: treatment conforming to the invention improved the filtration of the mash derived from the wort and lowered the ß-glucan content of the malt.

Example 4: PRODUCTION OF PREPARATIONS PRODUCED BY LACTIC ACID BACTERIA

1. Preparation of concentrated culture broths:

The production strains, Pediococcus pentosaceus VTT-E-90390 (E-390) and Lactobacillus plantarum VTT-E-78076 (E-76) were cultivated in a fermentor in a 15 l volume. Inoculum volume was 6% to 7%, culture was made on MRS medium, at 30°C, 2 days in microaerophilic conditions. The culture broths were concentrated tenfold and twenty-fold by lyophilizing and evaporating procedure. The microbicidic activity of the concentrates was ascertained by the disk method.

2. Preparation of a purified solution containing microbicidic compounds:

The lactic acid bacteria culture broth was purified by gel chromatographic fractionation according to molecule size. The fractions found by disk method and by turbidometry to be active were collected and rerun through the gel column.

Example 5: THE EFFECT OF LACTIC ACID BACTERIA PREPARATIONS AND OF PREPARATIONS PRODUCED BY LACTIC ACID BACTERIA ON THE MICROFLORA OF MALTING AND ON MALT QUALITY

1. Strains employed and preparations produced from the strains:

The following bacterial strains were used:

Lactobacillus plantarum VTT-E-78076 (E-76)

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Pediococcus pentosaceus VTT-E-90390 (E-390)

The strains were obtained from VTT, Collection of Industrial Microorganisms (Biotechnical Laboratory, Finland).

The preparations were made as described in Example 1, item 3., Example 3, item 1., and Example 4, items 1. and 2.

2. Barley:

The barley employed was Kymppi barley of the harvest year 1991.

3. Malting process:

Malting was carried out as described in Example 3, however with duration of the germination step, 8 days. The final moisture content of the malt came to be less than 5%.

4. Malting:

Two laboratory maltings were made. Malting without any additions served as control.

4.1 First malting:

Culture broths without cells, concentrated tenfold, and untreated culture broth including cells were used for preparations in the laboratory malting. Preparation was added at the beginning of steeping I or at the beginning of steepings I and II. Malting trials 1 to 8 were carried out as follows.

Test No. 1: Control, Kymppi barley 1991;
Test No. 2: At beginning of steepings I and II, 120 ml E-76 culture broth with cells is added:
Test No. 3: At beginning of steeping I, 120 ml tenfold concentrated E-76 culture filtrate is added;
Test No. 4: At beginning of steepings I and II, 120 ml tenfold concentrated E-76 culture filtrate is added;
Test No. 5: At beginning of steepings I and II, 120 ml E-390 culture broth is added;
Test No. 6: At beginning of steeping I, 120 ml tenfold concentrated E-390 culture filtrate is added;
Test No. 7: At beginning of steepings I and II, 120 ml

BNSDOCID: <WO___9416053A1_I_>

tenfold concentrated E-390 culture filtrate is added;
Test No. 8: At beginning of steepings I and II, 60 ml E-76 culture broth with cells and 60 ml E-390 culture broth with cells are added.

4.2. Second malting:

In the laboratory malting, twenty-fold concentrated culture filtrates without cells, purified microbicidic fractions without cells and untreated culture broths with cells were used for preparations. The steeping water pH was controlled. Preparation was added at the beginning of steepings I and II. Malting trials 9 to 16 were carried out as follows.
Test No. 9: Control, Kymppi barley 1991;
Test No. 10: At beginning of steepings I and II, 120 ml water is added, pH 3.8;
Test No. 11: At beginning of steepings I and II, 120 ml E-76 culture broth with cells is added;
Test No. 12: At beginning of steepings I and II, 120 ml E-76 fractionated concentrate is added, pH 3.8;
Test No. 13: At beginning of steepings I and II, 120 ml twenty-fold concentrated E-76 culture filtrate is added;
Test No. 14: At beginning of steepings I and II, 120 ml E-390 culture broth with cells is added;
Test No. 15: At beginning of steepings I and II, 120 ml E-390 fractionated concentrate is added, pH 3.8;
Test No. 16: At beginning of steepings I and II, 120 ml twenty-fold concentrated E-390 culture filtrate is added.

5. Analyses performed:

Samples were drawn from each malting step.

Moulds, lactic acid bacteria, total bacterial counts and physical and chemical quality characteristics of the malt were determined as described in Example 3.

6. Results:

Figures 4 and 5 show the total bacterial counts at different steps in the malting process on application

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of lactic acid bact ria culture broths with cells or of concentrated or fractionated culture filtrates in the steeping waters.

In Tables 7, 8, 9 and 10 are presented the concentrations of Fusarium moulds and other moulds, and of lactic acid bacteria in different malting steps, in both malting runs.

Tables 11 and 12 present the results of malt analysis from both malting runs.

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TABLE 7. Myco flora at malting on addition of P. pentosaceus E-390 and L- plantarum E-76 preparations to the barley steeping water (results given as % contaminated kernels). Numbering of samples in Example 5.

Sample	Bar-	Steeping								Germination								Malt							
Mould genus	ley	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Fusarium FP*)	35	80	64	60	22	76	76	48	68	72	88	88	94	96	80	90	94	26	22	2	3	25	17	7	24
CZID	36	74	72	46	38	84	54	32	78	84	82	80	82	84	78	80	96	36	22	10	2	24	20	8	24
Alternaria	26	6	18	22	20	18	22	12	24	18	10	8	2	10	2	2	10	18	8	17	5	18	5	11	16
Cephalosporium	37	22	38	34	36	42	12	28	24	72	20	42	80	42	32	36	24	2	3	5	4	7	2	6	2
Cladosporium	17	4	20	6	56	34	10	44	20	6	6	30	52	14	10	36	4	2	0	4	1	1	1	1	0
Rhitzopus	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	48	13	0	11	0
Mucor	0	2	4	0	2	8	14	0	4	0	10	28	64	24	44	34	14	65	79	78	88	68	83	96	73
Stemphylium	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	6	0	1
Epicoccum	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	1	1	0	0	3	0	1	3
Helminthosporium	10	26	46	10	10	36	16	14	30	48	64	60	42	50	54	38	58	28	26	14	3	11	23	8	20
Acremoniella	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Penicillium	3	0	2	2	2	0	0	2	2	4	2	2	0	2	2	2	0	1	0	0	0	0	0	1	0
Other fungi	1	8	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0

*) FP; filter paper

TABLE 8. Lactic acid bacteria counts. (CFU/ml) at malting on addition of P. pentosaceus E-390 and L. plantarum E-76 preparations to the barley steeping water. Numbering of samples in Example 5.

Example	Steeping	Germination	Malt
1	$5,5x10^3$	$3,3x10^6$	$2,5x10^2$
2	$7,3x10^6$	$1,2x10^7$	$5,5x10^5$
3	$1,4x10^3$	$4,4x10^3$	$1,1x10^3$
4	$3,2x10^2$	$1,4x10^4$	$9,5x10^2$
5	$1,3x10^7$	$7,5x10^6$	$2,6x10^6$
6	$4,8x10^3$	$5,0x10^5$	$4,0x10^4$
7	$7,3x10^2$	$2,4x10^4$	$2,6x10^3$
8	$1,2x10^7$	$1,4x10^7$	$2,3x10^6$

Lactic acid bacteria content of the barley: $7,5x10^2$ CFU/g.

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TABLE 9. Myco flora at malting on addition of P. pentosaceus E-390 and L- plantarum E-76 preparations to the barley steeping water (results given as % contaminated kernels). Numbering of samples in Example 5.

Sample	Bar-	Steeping								Germination								Malt							
Mould genus	ley	9	10	11	12	13	14	15	16	9	10	11	12	13	14	15	16	9	10	11	12	13	14	15	16
Fusarium FP*)	35	78	84	32	30	0	44	36	28	98	86	94	98	59	96	94	72	23	28	37	14	1	16	65	13
CZID	36	78	78	30	14	0	26	28	22	98	98	92	66	12	92	92	54	38	31	36	7	1	24	44	11
Alternaria	26	6	2	34	10	10	24	20	14	8	2	14	24	0	0	14	6	17	16	25	13	2	19	27	3
Cephalosporium	37	18	18	34	24	0	36	44	44	52	18	56	52	4	36	36	58	5	3	6	11	1	14	13	20
Cladosporium	17	6	8	14	16	70	18	24	42	14	6	2	16	54	2	6	20	1	0	0	1	7	1	0	1
Rhitzopus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	20	0	0	0	0	11	0
Mucor	0	0	2	2	2	4	2	0	2	36	14	52	40	76	22	56	66	80	78	78	91	98	93	84	90
Stemphylium	0	0	0	0	0	2	0	0	0	0	0	0	2	2	0	2	0	0	4	4	1	0	3	2	0
Epicoccum	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0
Helminthosporium	10	32	28	44	18	10	26	28	26	66	50	66	82	6	78	66	58	46	58	46	52	7	76	55	17
Acremoniella	0	0	0	0	0	0	0	0	0	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
Aspergillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium	3	2	2	0	2	0	0	2	0	0	0	10	16	30	4	4	10	0	1	0	0	0	0	0	0
Other fungi	1	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0

*) FP; filter paper

TABLE 10. Lactic acid bacteria counts. (CFU/ml) at malting on addition of P. pentosaceus E-390 and L. plantarum E-76 preparations to the barley steeping water. Numbering of samples in Example 5.

Example	Steeping	Germination	Malt
9	$8,0x10^2$	$4,2x10^6$	$8,0x10^3$
10	$8,0x10^2$	$5,0x10^6$	$5,6x10^4$
11	$2,7x10^7$	$2,1x10^7$	$8,5x10^5$
12	$2,2x10^3$	$3,4x10^5$	$2,5x10^3$
13	$1,2x10^3$	$1,4x10^7$	$2,3x10^3$
14	$4,1x10^7$	$3,5x10^7$	$1,1x10^4$
15	$4,8x10^3$	$4,8x10^6$	$3,9x10^5$
16	$3,5x10^2$	$2,1x10^5$	$1,7x10^4$

Lactic acid bacteria content of the barley: $7,5x10^2$ CFU/g.

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TABLE 11. Effect of P. pentosaceus E-390 and L. plantarum E-76 preparations added to the barley steeping water on malt quality. Numbering of samples in Example 5.

Analysis		Sample							
		1	2	3	4	5	6	7	8
Moisture	%	3,6	3,6	3,5	3,7	3,7	3,6	3,9	3,9
Extract content, fine	% dry m.	81	80,8	80,6	80	81,2	81	80,7	81,2
Extract content, coarse	% dry m.	76,8	77	76,1	75,2	77	76,4	74,8	77,1
Extract difference	%	4,2	3,8	4,5	4,9	4,3	4,7	5,9	4,1
Saccharification	min.	<10	<10	<10	<10	<10	<10	<10	<10
Wort clarity		clear	clear	clear	clear	clear	clear	clear	clear
Filtration, fine grind	ml/min	320/45	320/44	315/48	315/40	320/47	315/66	320/49	315/52
Filtration, coarse grind	ml/min	260/86	265/64	270/59	255/73	260/68	260/76	255/90	275/120
Wort	pH	5,93	5,88	5,86	5,86	5,86	5,86	5,97	5,99
Viscosity	cP	1,69	1,57	1,59	1,57	1,55	1,58	1,59	1,58
ß-glucans	mg/l	784	659	705	736	610	696	822	657
Free amino nitrogen	mg/l	154	156	155	139	158	150	130	154
Modification	%	74	72	73	73	72	75	77	76
Homogeneity	%	61	65	63	58	67	66	62	68
α-amylase	U/g	178	182	148	128	179	156	144	174
ß-glucanase	U/kg	434	420	360	360	442	412	361	425

SUBSTITUTE SHEET

BNSDOCID: <WO___9416053A1_I_>

SUBSTITUTE SHEET

TABLE 12. Effect of P. pentosaceus E-390 and L. plantarum E-76 preparations added to the barley steeping water on malt quality. Numbering of samples in Example 5.

Analysis		Sample							
		9	10	11	12	13	14	15	16
Moisture	%	3,5	3,6	3,6	3,5	3,8	3,5	3,5	3,6
Extract content, fine	% dry m.	80,5	80,5	81,2	80,5	80,3	81	81,1	80,4
Extract content, coarse	% dry m.	78,4	77,9	77,4	73,3	73,5	77,6	76,6	75,1
Extract difference	%	2,1	2,6	3,7	7,2	6,8	3,4	4,5	5,4
Saccharification	min.	<10	<10	<10	<10	<10	<10	<10	<10
Wort clarity		clear	clear	clear	clear	clear	clear	clear	clear
Filtration, fine grind	ml/min	320/40	315/47	320/44	315/42	320/55	315/49	330/40	320/40
Filtration, coarse grind	ml/min	290/120	270/76	260/51	265/120	245/120	285/68	305/120	265/12
Wort	pH	6,04	6,05	5,99	5,97	5,94	5,95	5,98	5,99
Viscosity	cP	1,54	1,65	1,51	1,53	1,5	1,49	1,49	1,52
ß-glucans	mg/l	442	522	397	553	612	381	463	576
Free amino nitrogen	mg/l	149	145	149	128	117	149	139	120
Modification	%	85	85	86	82	74	81	75	71
Homogeneity	%	63	66	76	57	57	68	51	47
α-amylase	U/g	161	172	161	123	72	148	125	101
ß-glucanase	U/kg	365	375	365	282	282	371	342	308

The results now obtained reveal that treatments according to the invention reduce, in particular, the Fusarium mould quantity and the total bacterial count in the different malting steps. It is further noted that, in addition to culture broths, concentrated and fractionated culture filtrates in particular have a favourable effect e.g. as regards Fusarium moulds.

From the malt analyses the inference can be drawn that culture broth additions of strains E-76 and E-390 had an effect of improving the filtrability of the wort made from the malt; the ß-glucan contents are also lower in the respective malts than in the control malt.

EXAMPLE 6: THE EFFECT ON MASH FILTRATION OF PREPARATIONS PRODUCED BY LACTIC ACID BACTERIA, ADDED TO THE MALTING

In Fig. 6 a diagram is reproduced, showing the effect of treatment according to the invention with preparations derived from lactic acid bacteria strains (120 ml culture broth per kg of barley) on the filtration of the mash produced using malt thus treated.
The strains mentioned in Example 1 were used in this experiment: E-390, E-416, E-98, E-317, E-390, E-76,, and E-315. The test was carried out using the Tepral filtration method (BIOS. 19, 1988, Grandclerc, J. et al.,, "Simplification de la méthode de filtration du brassin tepral description de la méthode", p. 88-92).

It is evident from the results that treatment according to the invention improves the filtration of the mash.

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 10, line 29.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM)

Address of depositary institution *(including postal code and country)*

Mascheroder Weg 1B
D-3300 Braunschweig
Germany

Date of deposit	Accession Number
15 January 1993	7388

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [X]

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Accession Number: 7388

C. ADDITIONAL INDICATIONS

In respect of Australia, the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

In respect of Netherlands, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert nominated by the requester.

In respect of Switzerland, the furnishing of samples to a third party may be subjected to the condition that that party indicates to the depositary institution its name and address for the purpose of information of the depositor and undertakes:
a) not to make available the deposited culture or a culture derived from it to a third party;
b) not to use the culture outside the purview of the law;
c) to produce, in case of a dispute, evidence that the obligations under items (a) and (b) have not been violated.

In respect of Denmark, Finland, Norway, Sweden and United Kingdom, the furnishing of a sample shall only be effected to an expert in the art.

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 10, line 26

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM)

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1B
D-3300 Braunschweig
Germany

Date of deposit	Accession Number
15 January 1993	7389

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☒

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Accession Number: 7389

C. ADDITIONAL INDICATIONS

In respect of Australia, the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

In respect of Netherlands, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert nominated by the requester.

In respect of Switzerland, the furnishing of samples to a third party may be subjected to the condition that that party indicates to the depositary institution its name and address for the purpose of information of the depositor and undertakes:
a) not to make available the deposited culture or a culture derived from it to a third party;
b) not to use the culture outside the purview of the law;
c) to produce, in case of a dispute, evidence that the obligations under items (a) and (b) have not been violated.

In respect of Denmark, Finland, Norway, Sweden and United Kingdom, the furnishing of a sample shall only be effected to an expert in the art.

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

BNSDOCID: <WO___9416053A1_I_>

CLAIMS

1. A procedure for treatment of seed material to be germinated, characterized in that to the seed material is added, in connection with the germination process, a lactic acid bacteria preparation or a preparation produced by lactic acid bacteria which has an effect inhibiting microbial growth.

2. Procedure according to claim 1, characterized in that the lactic acid bacterium belongs to genus Lactococcus, Leuconostoc, Pediococcus or Lactobacillus.

3. Procedure according to claim 1 or 2, characterized in that the lactic acid bacteria preparation or the preparation produced by lactic acid bacteria has been derived from species Lactococcus lactis, Leuconostoc mesenteroides, Pediococcus damnosus, Pediococcus parvulus, Pediococcus pentosaceus, Lactobacillus curvatus or Lactobacillus plantarum, or from a mixture of these, advantageously from species Lactobacillus plantarum or Pediococcus pentosaceus or from a mixture of these.

4. Procedure according to any one of claims 1-3, characterized in that to barley kernels is added in connection with a malting process, a lactic acid bacteria preparation or a preparation produced by lactic acid bacteria having an effect inhibiting the growth of Fusarium moulds.

5. Procedure according to claim 4, characterized in that the lactic acid bacteria preparation or the preparation produced by lactic acid bacteria is added in the steeping or germination step.

6. Procedure according to any one of claims 1-5, characterized in that the lactic acid bacteria preparation or the preparation produced by lactic acid bacteria is added to seed material going to be converted to sprouts intended to serve as nutrition.

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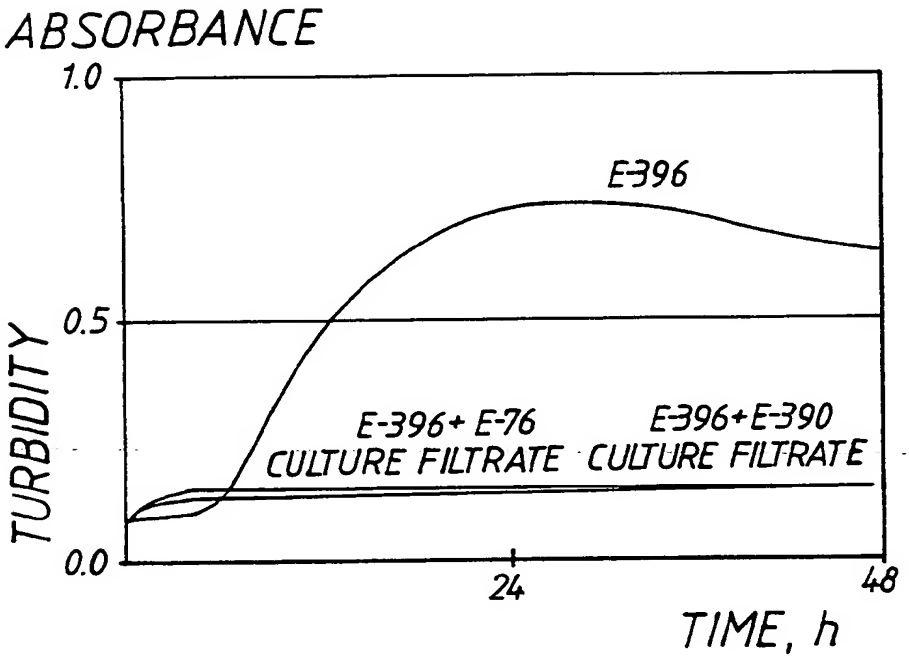

Fig. 1

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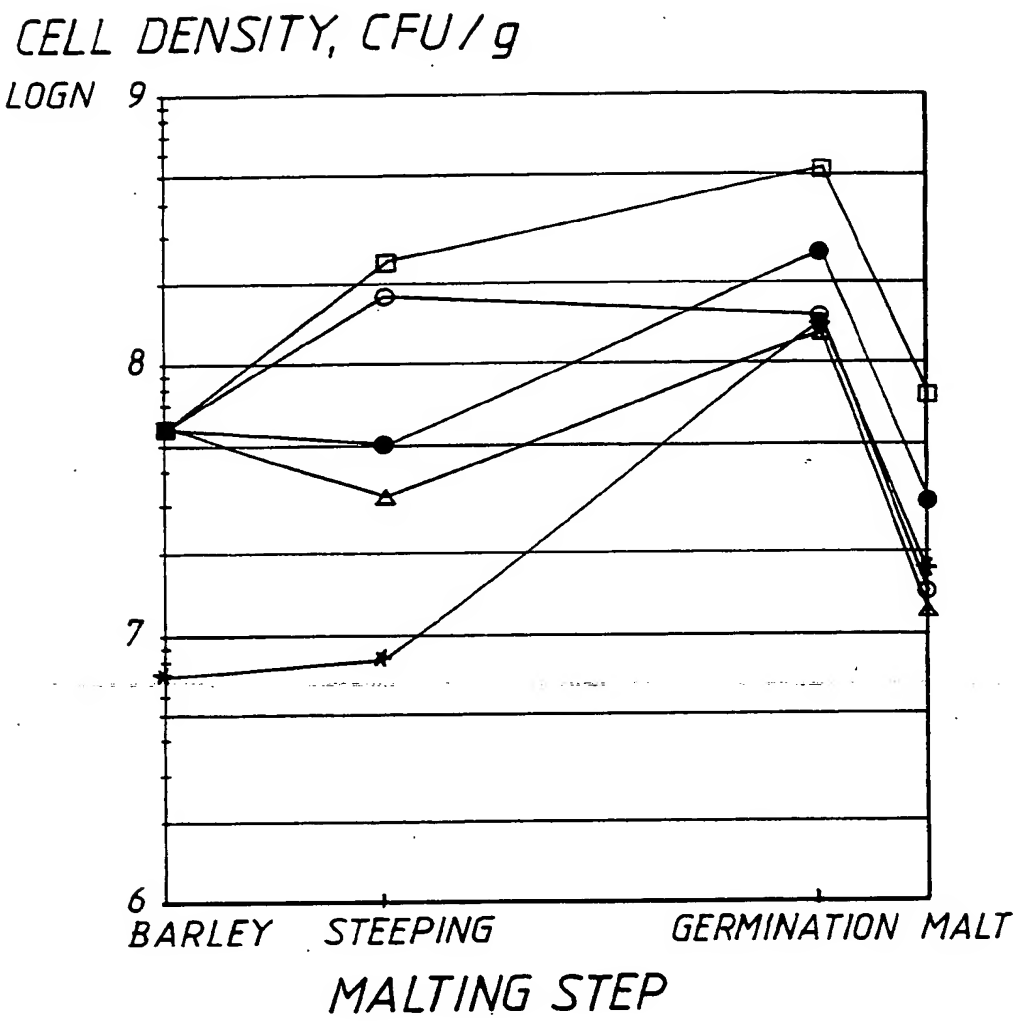

Fig. 2

BNSDOCID: <WO___9416053A1_I_>

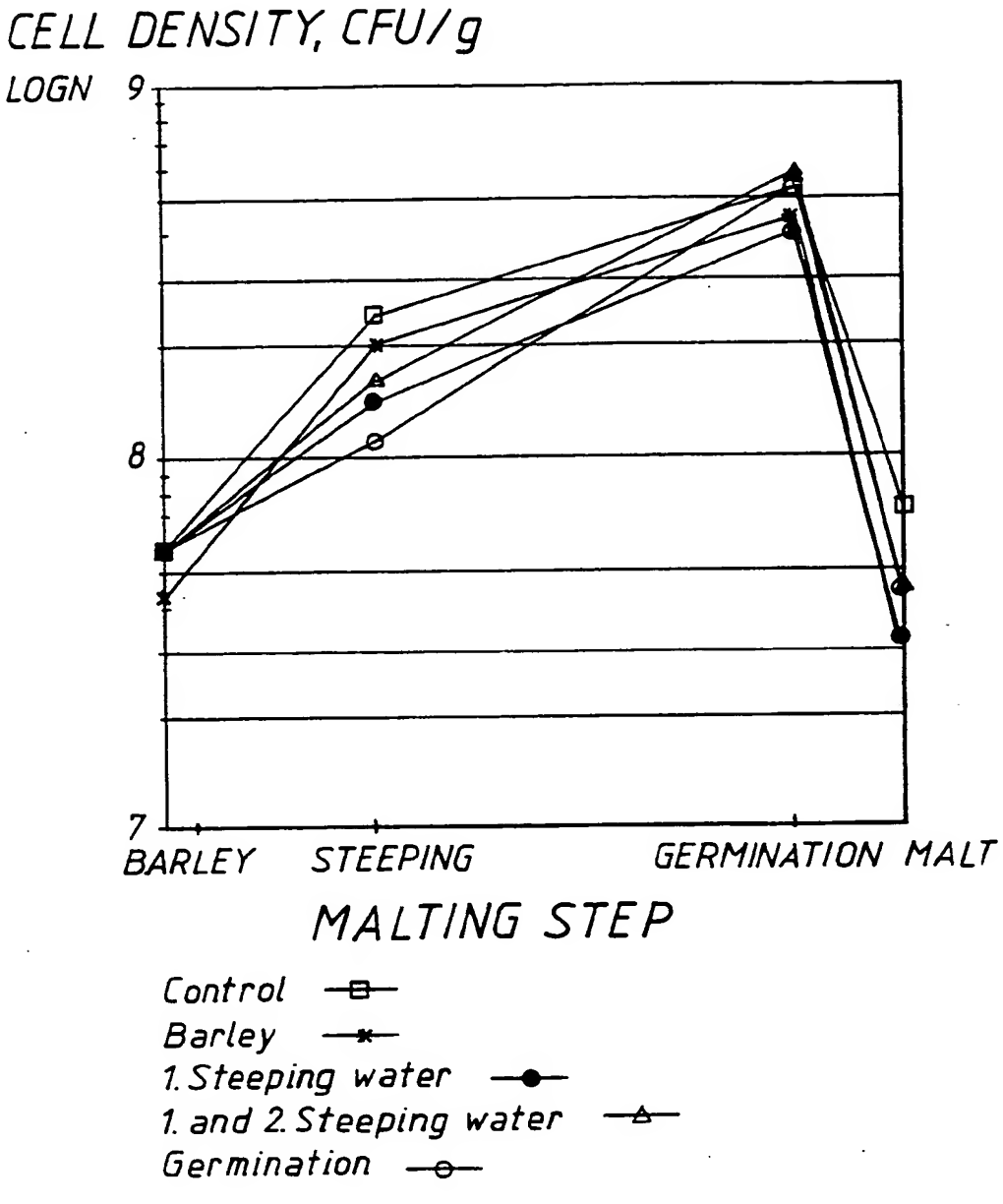

Fig. 3

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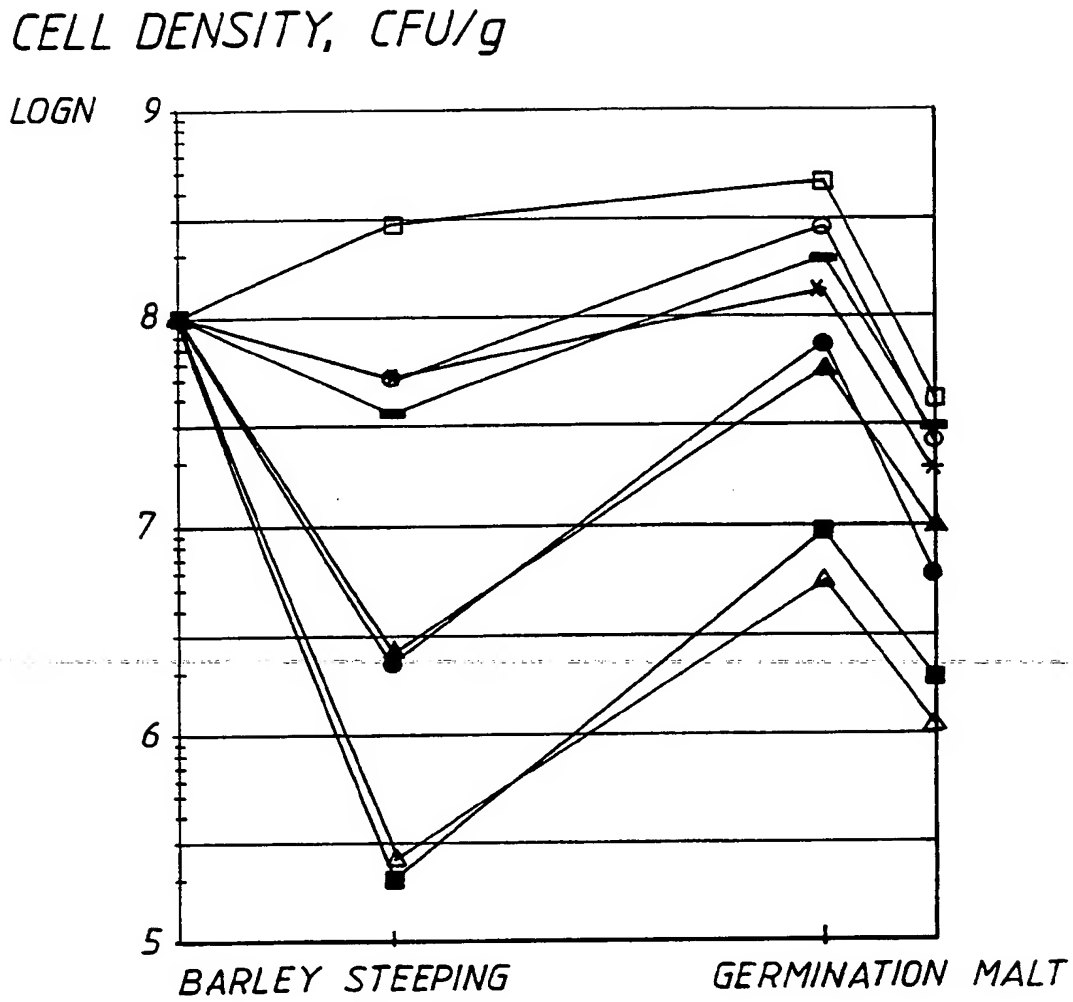

Fig.4

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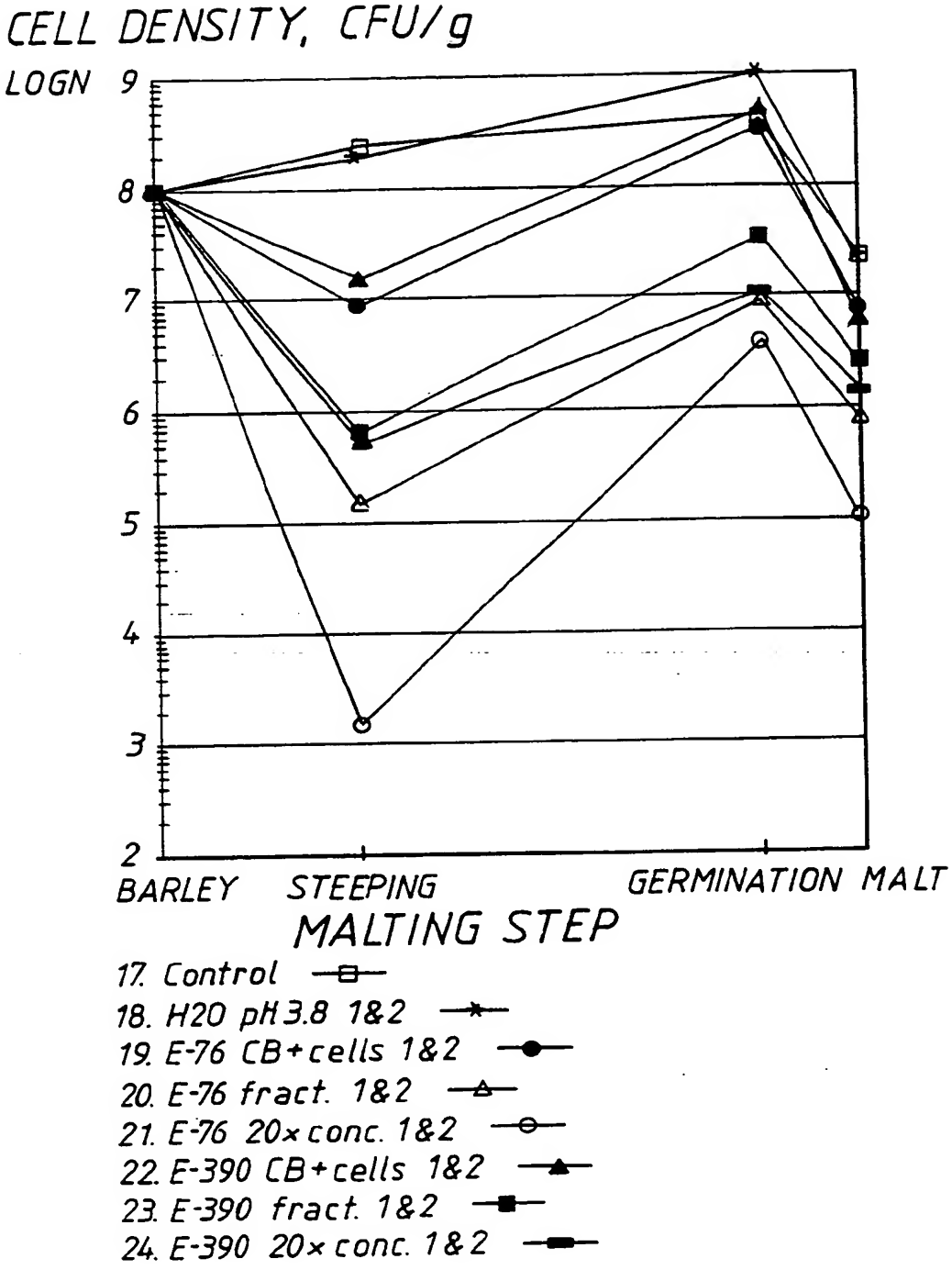

Fig.5

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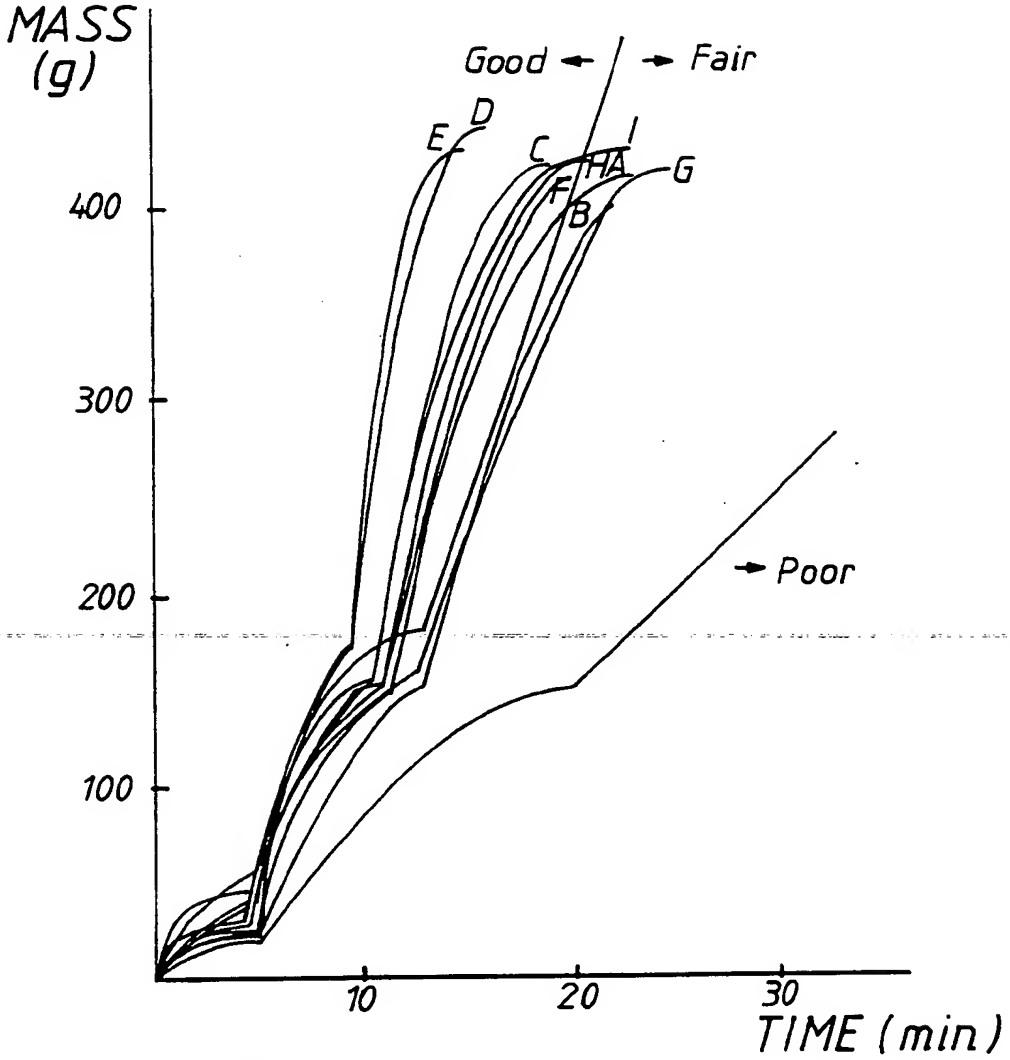

A: Control
B: Control
C: 120 ml E-390 culture broth to germination
D: 120 ml E-390 culture broth to 1. and 2. steeping water
E: 120 ml E-416 culture broth to 1. and 2. steeping water
F: 120 ml E-76 culture broth to germination
G: 120 ml E-98 culture broth to germination
H: 120 ml E-315 culture broth to germination
I: 120 ml E-317 culture broth to germination

Fig. 6

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 93/00388

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12C 1/02, C12C 1/00, C12C 1/04, A23L 1/172
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, CA, WPI, CLAIM, DERWENT BIOTECHNOLOGY ABS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB, A, 1543463 (O. MOEBUS), 4 April 1979 (04.04.79), page 2, line 22 - line 31, examples --	1-6
X	US, A, 2903399 (T. R. DIXON), 8 Sept 1959 (08.09.59) -- --------	1-6

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 April 1994	05 -05- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Jonny Brun Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT
Information on patent family members

26/02/94

International application No.
PCT/FI 93/00388

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 1543463	04/04/79	AU-B- 510281	19/06/80
		AU-A- 2821577	08/03/79
		DE-A,B,C 2638163	02/03/78
		FR-A- 2362596	24/03/78
		JP-A- 54122739	22/09/79
		NL-A- 7709398	28/02/78
		SE-A- 7709396	26/02/78
		DE-A,B,C 2641270	16/03/78
		DE-A,B,C 2645480	13/04/78
US-A- 2903399	08/09/59	NONE	

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